Remarks

I. Disposition of the Claims

Claims 1-16, 18, 20, 23, and 23-33 are withdrawn from consideration.

Claims 17 and 19 have been amended in this response to remove the phrase that indicates that L "optionally can be included in a ring." Applicants have also amended Claims 17 and 19 to indicate that detectable biomolecules of formula B-(-L-(D)m)n have a molar extinction coefficient of at least about 40,000 M^{-1} cm^{-1}. Support for this amendment can be found on page 10, lines 14-16 of the specification.

In addition, Claim 17 has been amended to clarify that the biological system in step (a) includes a visually detectable biomolecule.

Claim 19 has been amended to correct a typographical error in the formula of the visually detectable biomolecule formed in step a). Formula B-(-L-$(P)_m)_n$ has been replaced with formula B-(-L-$(D)_m)_n$. Support for this amendment can be found on page 8, lines 3-15 of the specification

Claim 19 has also been amended to indicate that "X" reacts with a biomolecule to form an ester, amide, phosphate, phosphorothioate, phosphonate, thioester or disulfide bond to form a compound having the formula B-(-L-$(D)_m)_n$. Support for this amendment can be found on page 4, lines 9-16 of the specification.

II. Rejection of Claims 17, 19, 21-22 and 24 Under 35 U.S.C. §112, Second Paragraph

A. The Definition of "L"

The Examiner states that the phrase "wherein the linear atoms in L . . optionally can be included in a ring" is vague and indefinite. The Examiner requests that Applicants clarify this definition.

Applicants have amended Claims 17 and 19 to remove the phrase "optionally can be included in a ring" from the definition of "L". In Claims 17 and 19, as amended, "L" is defined as a spacer group that comprises from one to about 10 linear atoms. This phrase is intended to mean that "L" contains from one to about 10 atoms that are linked together in a chain. Claims 17 and 19 also state that "L can be optionally substituted". Applicants intend the phrase "L can be

optionally substituted" to include the condition wherein substituents on two linear atoms of L are connected to each other to form a ring.

B. Unsubstituted Perylenyl

The Examiner has stated that the phrase "provided that D is not unsubstituted perylenyl" is vague and indefinite because the Examiner believes that perylenyl would necessarily have to be substituted with a linker in order to bind to the biomolecule.

Applicants disagree with the Examiner's assertion that the phrase "provided that D is not unsubstituted perylenyl" is vague and indefinite. Applicants' formula for a visually detectable biomolecule in Claim 17 and a reactive dye in Claim 19 are as follows:

Visible Detectable Biomolecule: $B-(-L-(D)_m)_n$

Reactive Dye: $(D)_n-L-X$

Both formulas indicate that D is a group that is a substituent on L. Applicants have indicated that one group that D cannot be is an unsubstituted perylenyl. According to chemical nomenclature, the suffix "yl" added to the end of perylene indicates that the group is a substituent attached, as indicated in the formula, to L. The adjective "unsubstituted" that modifies the perylenyl group indicates that the perylenyl substituent is not itself substituted.

Applicants have used these terms as they are generally accepted and used by those skilled in the art. For example, in EP 0 808 829 B1, cited by the Examiner, R^1 and R^2 are defined as a substituted or unsubstituted aryl (see EP 0 808 829 B1, page 3, lines 51-54). The "yl" suffix indicates that the aryl is a substituent attached to a nitrogen, as shown in formula II of EP 0 808 829 B1, and that the aryl group can be otherwise unsubstituted. Thus, Applicants usage of the phrase "unsubstituted perylenyl" is in accordance with generally accepted chemical nomenclature and is not indefinite. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

III. Rejection of Claims 17, 19, 21-22 and 24 Under 35 U.S.C. §102(b) Over Bevers, *et al.*, *J. Am. Chem. Soc.* (1998), *120*:11004-11005 (hereinafter "Bevers")

The Examiner states that Bevers discloses a method of tethering a perylene bisimide to DNA, then detecting the biomolecule during melting studies using absorption measurements. The Examiner takes the position that Bevers anticipates Applicants' method of Claims 17, 19, 21-22 and 24.

Applicants disagree with the Examiner's conclusion that Bevers anticipates method Claims 17, 19, 21-22 and 24. Bevers discloses compounds that have a 3,4,9,10-perylene diimide linking two pyrimidine DNA strands (Bevers, page 11004, Col. 1, paragraph 2). Table 1 on page 11005 shows that 3,4,9,10-perylene diimide is linked to two DNA molecules.

Applicants' method of Claims 17 and 19 require that the biomolecule being detected has the following formula:

$$B\text{-}(\text{-}L\text{-}(D)_m)_n$$

As can be seen from the above formula, one biomolecule (represented by "B" in the formula) is attached to one to about 5 linkers (represented by "L") and each linker includes one to about 5 visible dye substituents (represented by "D"). In addition, Applicants' Claims 17 and 19 state that the photostable visible dye, represented by "D", has only one linkage to a biomolecule.

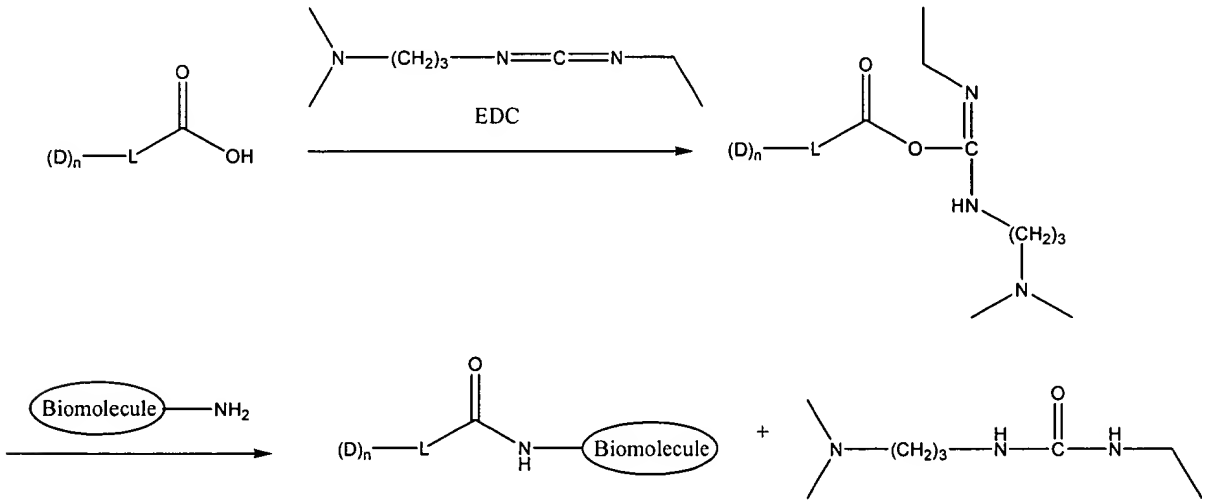
Since Applicants' method requires that only one biomolecule is linked to a visible dye molecule and Bevers disclosed two DNA molecules linked to a perylene molecule, Applicants method of Claims 17 and 19, and the claims depending therefrom, is not anticipated by Bevers. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

IV. Rejection of Claims 17, 19, 21-22 and 24 Under 35 U.S.C. §102(b) Over Suzuki, et al., EP 0 808 829 (hereinafter "Suzuki")

The Examiner states that Suzuki discloses a nucleic acid detection method in which a polycyclic fluorescent labels are covalently attached to the nucleic acid via a reactive carbodiimide group and a straight chain linker. The Examiner takes the position that Suzuki anticipates Applicants' method of Claims 17, 19, 21-22 and 24.

Applicants disagree with the Examiner's conclusion that Suzuki anticipates method Claim 17 and also assert that Suzuki does not anticipate Claim 19, as amended. Suzuki discloses fluorescent group-containing carbodiimide compounds, wherein the carbodiimide portion of the molecule reacts with a nucleic acid base to form a bond. Suzuki also discloses that proteins will react with the carbodiimide group to form a bond (Suzuki, page 18, paragraph 0067). Suzuki does not disclose what type of bond is formed between the carbodiimide compound and nucleic acid or protein.

Applicants' Claim 17 and Claim 19, as amended, indicate that the spacer group "L" is attached to the biomolecule via an ester, amide, phosphate, phosphorothioate, phosphonate, thioester or disulfide linkage. Although Suzuki does not disclose what type of bond is formed between the carbodiimide group, it is unlikely to be an ester, amide, phosphate, phosphorothioate, phosphonate, thioester or disulfide linkage. Applicants disclosed on page 19, lines 6-10 of the specification the use of a 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide (EDC) as a coupling agent to form an amide or ester bond between an amine or hydroxyl, respectively, and a carboxyl group. A coupling reaction using EDC proceeds as follows:



As can be seen from the above scheme, no part of the carbodiimide coupling agent (EDC) used by Applicants becomes attached to the biomolecule.

Unlike Applicants' use of EDC as a coupling reagent, Suzuki discloses fluorescent group-containing carbodiimide compounds that become attached to the biomolecule, thus labeling the biomolecule with a fluorescent label. However, Suzuki does not disclose that the fluorescent label is attached to a biomolecule via an ester, amide, phosphate, phosphorothioate, phosphonate, thioester or disulfide linkage. Since Suzuki does not disclose all of the limitations

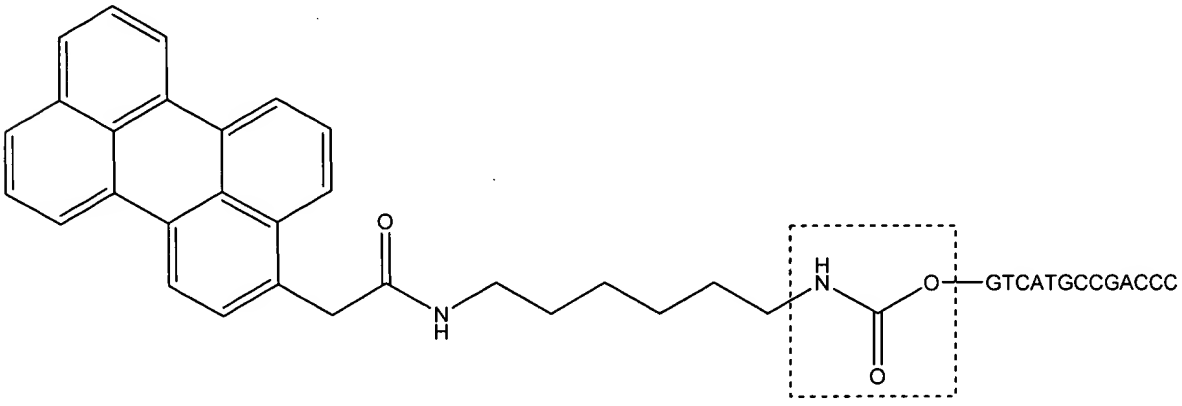
of Applicants' claimed method, Suzuki does not anticipate Applicants' method of Claims 17 and 19 and the claims depending therefrom. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

V. Rejection of Claims 17, 19, 21-22 and 24 Under 35 U.S.C. §102(b) Over Balakin, *et al.*, *Biosensors & Bioelectronics* (1998), *13*:771-778 (hereinafter "Balakin")

In a telephone conference between Theresa A. Devlin and Examiner Epperson on January 26, 2004, the Examiner indicated that the reference cited in the Office Action as Balakin, et al. (EP 0 808 829) was supposed to be Balakin, *et al.*, *Biosensors & Bioelectronics* (1998), *13*:771-778. Applicants thank the Examiner for sending the appropriate reference to them via facsimile.

The Examiner states that Balakin discloses conjugates of pyrene and perylene with oligodeoxynucleotides for the detection of said oligodeoxynucleotides. The Examiner states that a reactive ester is used to attach the fluorophore to the nucleic acid. The Examiner concludes that Balakin anticipates Applicants' Claims 17, 19, 21-22 and 24.

Applicants disagree with the Examiner's conclusion that Balakin anticipates method Claims 17, 19, 21-22 and 24. Balakin introduces a perylene label to a 5'-aminoalkylated oligonucleotide (see Balakin, page 773, Fig. 2) to form a labeled oligonucleotide having the following structure:

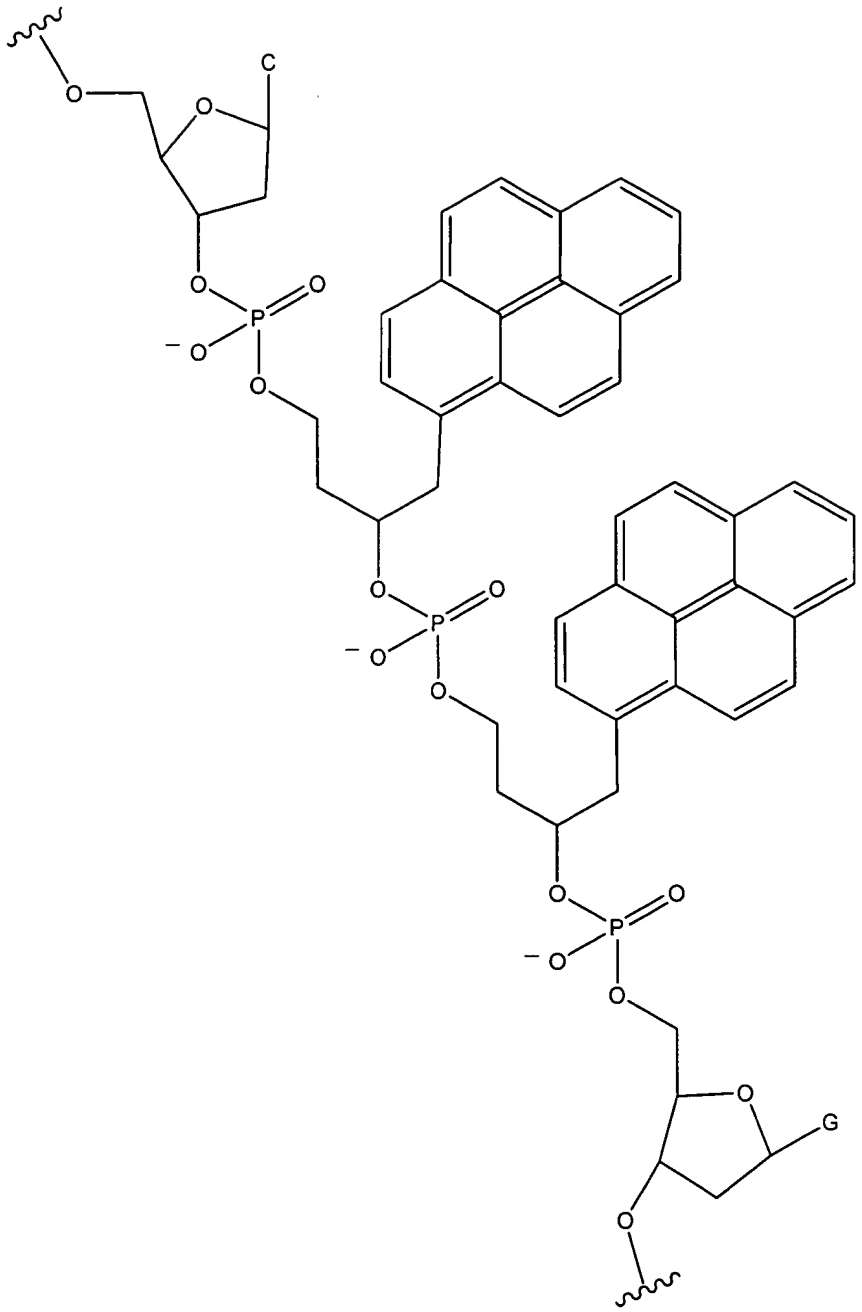


Compound 6 of Balakin

Compound 6 prepared by Balakin differs from the visually detectable biomolecules used in Applicants' method in at least two ways. First, Applicants' Claims 17 and 19 indicate that the photostable visible dye radical represented by "D" which is attached to the spacer "L" in the formula for a visually detectable biomolecule, $B-(-L-(D)_m)_n$, cannot be an unsubstituted perylenyl group. The dye radical in Compound 6 of Balakin is an unsubstituted perylenyl group which is not encompassed by Applicants' claims.

In addition, Balakin's method, which utilizes Compound 6, is not encompassed by Applicants' Claim 17 and Claim 19, as amended, because the oligonucleotide is attached to the spacer molecule via a carbamate linkage. Applicants' Claim 17 and Claim 19, as amended, require that the spacer group is attached to the biomolecule via an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage. Thus, Balakin's labeling method which utilizes compound 6 is not encompassed by Applicants' Claims 17, 19 and the claims depending therefrom.

Balakin discloses other compounds in addition to compound 6. The use of these compounds also does not anticipate Applicants' claimed method. For example, Balakin discloses oligonucleotides that are labeled in the middle of the oligonucleotide (see Balakin, page 774, Table 1, compounds 21-23, and Fig. 5). Conjugates in which the pyrenyl group is in the middle of the oligonucleotide have the following structure:



Segment of Compound 22

As can be seen from Compound 22, the spacer is attached to two oligonucleotide molecules. Balakin's compounds 21 and 23 have a similar structure where the pyrenyl groups are attached through a spacer to two oligonucleotide molecules (see Balakin, page 774, Table 1).

Applicants' method of Claims 17 and 19 require that the biomolecule being detected has the following formula:

$B\text{-}(\text{-}L\text{-}(D)_m)_n$

As can be seen from the above formula, only one biomolecule (represented by "B" in the formula) is attached to one to about 5 linkers (represented by "L") and each linker includes one to about 5 visible dye substituents (represented by "D").

Since Applicants' method requires that only one biomolecule is linked to the spacer containing a visible dye molecule and Balakin's Compound 21-23 has a spacer containing the dye molecules attached to two oligonucleotides, Applicants method of Claims 17 and 19, and the claims depending therefrom, is not anticipated by any of Balakin's compounds 21-23 that have the dye molecules in the middle of the oligonucleotide strand.

Another set of compounds disclosed by Balakin are labeled at the 3' or 5' end of the oligonucleotide with a pyrenyl group (see Balakin, page 774, Table 1, compounds 10-12, 14, 16, 17, and 19). Balakin discloses fluorescent emission spectra for these compounds in Figs. 7, 8, 9, 10, and 11. As can be seen from these figures, Compounds 10-12, 14, 16, 17, and 19 have emission peaks that are less than 400 nm. Typically, dyes absorb at a wavelength that is shorter than the wavelength at which they emit radiation. Therefore, the absorbance peaks for Balakin's Compounds 10-12, 14, 16, 17, and 19 most likely have an even shorter wavelength than 400 nm.

Applicants' Claims 17 and 19 require that the biomolecule formed in the method must be visually detectable. According to Applicants' specification, "visually detectable" substances absorb and emit light in a region of the spectrum ranging from about 400 nm to about 800 nm (see page 10, lines 11-14 of the specification).

Since Compounds 10-12, 14, 16, 17, and 19 do not have emission maxima or absorbance maxima in the wavelength range that Applicants define as the visible range, Balakan's use of Compounds 10-12, 14, 16, 17, and 19 does not anticipate Applicants' method of Claims 17 and 19, and the claims depending therefrom.

Since none of the compounds disclosed by Balakin meet all of the limitations of the compounds used in Applicants' claimed method, Balakin does not anticipate Applicants' Claims 17 and 19, and the claims depending therefrom. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

VI. Rejection of Claims 17, 19, 21-22 and 24 Under 35 U.S.C. §102(b) Over Kool, U.S. Patent No. 6,479,650 (hererinafter "Kool"

The Examiner states that Kool discloses a method for visually detecting nucleic acids using fluorescent cyclic compounds joined to a carbon of a sugar molecule. The Examiner takes the position that Kool anticipates Applicants' method of Claims 17, 19, 21-22 and 24.

Kool discloses a method of fluorescent labeling of nucleic acids by replacing one or more DNA or RNA bases with a fluorescent cyclic compound. The compounds disclosed by Kool have a sugar moiety with a fluorescent cyclic group attached thereto. Kool discloses that perylene imide and perylene amide can be used as the fluorescent cyclic group (see Kool, Col. 5, lines 29-46). The sugar moiety of the compounds disclosed by Kool can be derivatized with a phosphoramidate group (see Kool, Col. 23, lines 51-59). Kool prepares several fluorescently labeled biomolecules (see Kool, 31, Table 1). Although biomolecule 3c disclosed by Kool has a molar extinction coefficient of 40,100 M^{-1} cm^{-1}, generally the biomolecules disclosed by Kool have molar extinction coefficients of less than 35,000 M^{-1} cm^{-1} (see Kool, Col. 31, Table 1).

Applicants' Claims 17, 19, 21-22 and 24 are directed to a method for visually detecting a biomolecule. The visually detectable biomolecules of Applicants' method of Claims 17 and 19, as amended, must have a molar extinction coefficient of at least 40,000 M^{-1} cm^{-1}. In addition, the visually detectable biomolecules of Applicants' method must absorb and emit light in a region of the spectrum ranging from about 400 nm to about 800 nm.

Applicants' Claims 17 and 19, as amended, differ from the method disclosed by Kool because the visually detectable biomolecules of Applicants' method have a molar extinction coefficient of at least 40,000 M^{-1} cm^{-1}, whereas the fluorescently labeled biomolecules disclosed by Kool generally have molar extinction coefficients of less than 35,000 M^{-1} cm^{-1}. The one exception is biomolecule 3c. However, biomolecule 3c has an absorption maximum of 285 nm and an emission maximum of 345 nm. Therefore, biomolecule 3c of Kool does not absorb and emit light in the region of the spectrum ranging from about 400 nm to about 800 nm, and thus, is not visually detectable as defined in Applicants' specification.

Since none of the biomolecules used in the method disclosed by Kool have both a molar extinction coefficient of 40,000 M^{-1} cm^{-1} and absorb and emit light in the visually detectable region of the spectrum as defined by Applicants' specification, Applicants method of Claims 17

and 19, and the claims depending therefrom, are not anticipated by Kool. Therefore, Applicants respectfully request that the rejection be reconsidered and withdrawn.

Please charge any fees associated with this filing, or apply any credits, to our Deposit Account No. 03-1721.

Respectfully Submitted,

Dated: 2/11/04

Theresa A. Devlin
Registration No. 45,361

Choate, Hall & Stewart
Exchange Place
53 State Street
Boston, MA 02109
(617) 248-5000